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Drinking Water Criteria Document for Total Coliforms (Final Draft)

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16. ABSTRACT

The Office of Drinking Water, U.S. Environmental Protection Agency, has prepared a drinking water criteria document on total coliforms. This document includes an extensive review of the following topics: waterborne disease agents, utility of total coliforms as an indicator for treatment efficiency, deterioration of the disinfection system, and fecal contamination, analytical procedures, and risk assessment.

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I. INTRODUCTION

During 1971-1981, there were 347 reported outbreaks of waterborne disease in the United States, involving 82,404 cases (CDC, 1982b). The annual number of outbreaks and cases from 1946-1980 is shown in Figure I-1 and I-2, respectively (Lippy and Waltrip, 1984). The waterborne disease surveillance system is generally a passive one, and there is ample evidence to suggest the values in Figure I-1 and I-2 represent but a small fraction of the outbreaks and cases which occur each year (CDC, 1982b). This is supported by the evidence that only four states reported 59 percent of all of the 1981 outbreaks (CDC, 1982b). Three of these four states (Colorado, Washington, and Vermont) received federal funds for surveillance from EPA in 1981, while the fourth, Pennsylvania, already has an extremely efficient surveillance system. Colorado received USEPA funds in 1980 and 1981, and reported an average number of outbreaks of 7 per year, while in 1971-1979, before funding, the average number of outbreaks was only 1.9 per year (CDC, 1982b).

One reason for the under-reporting is that waterborne diseases are often mild and self-limiting, and consequently an outbreak may not be recognized.

FIG. I-1. NUMBER OF WATERBORNE OUTBREAKS, 1946 - 1980

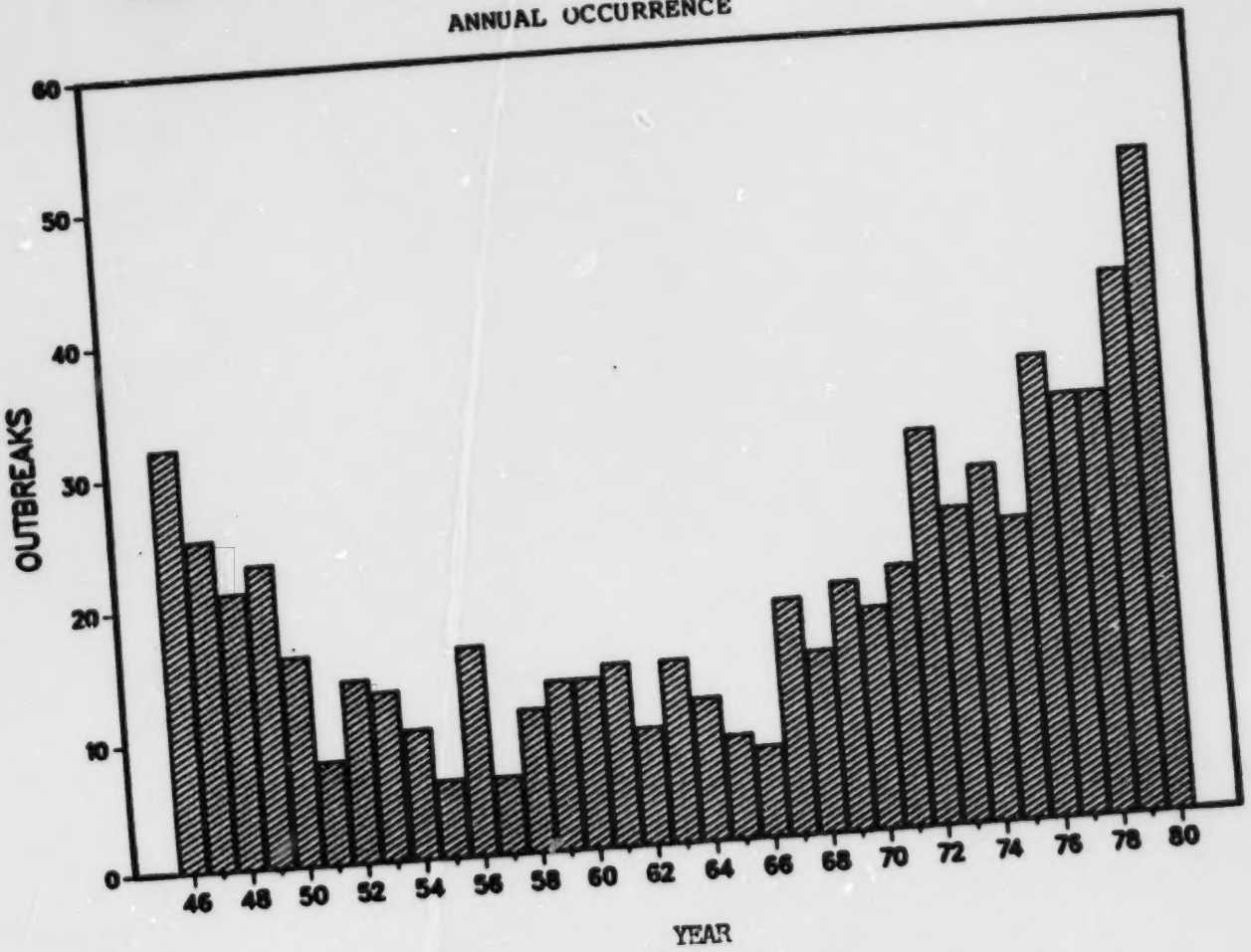
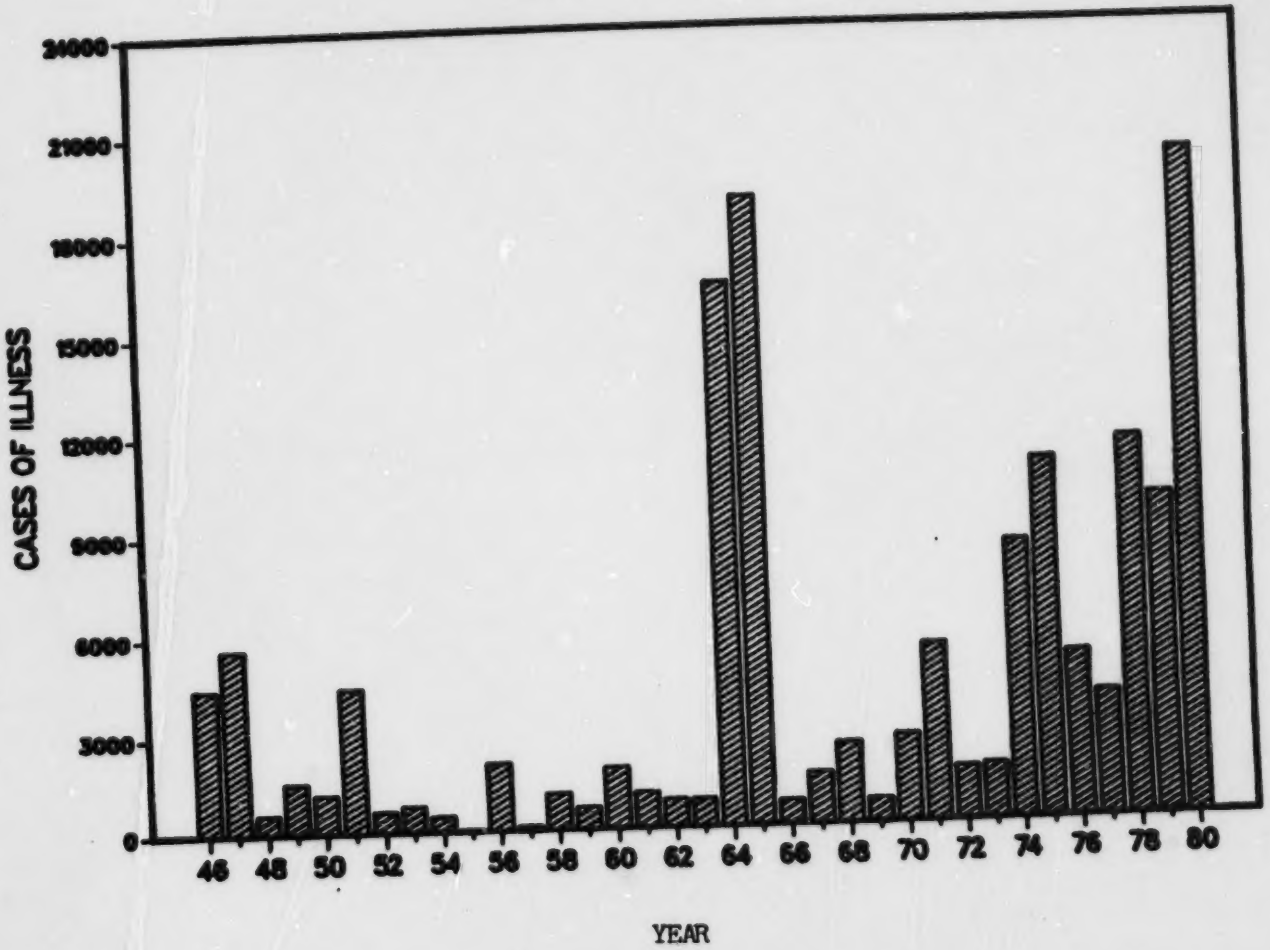


Fig. I-2

WATERBORNE OUTBREAKS, 1946–1980
CASES OF ILLNESS



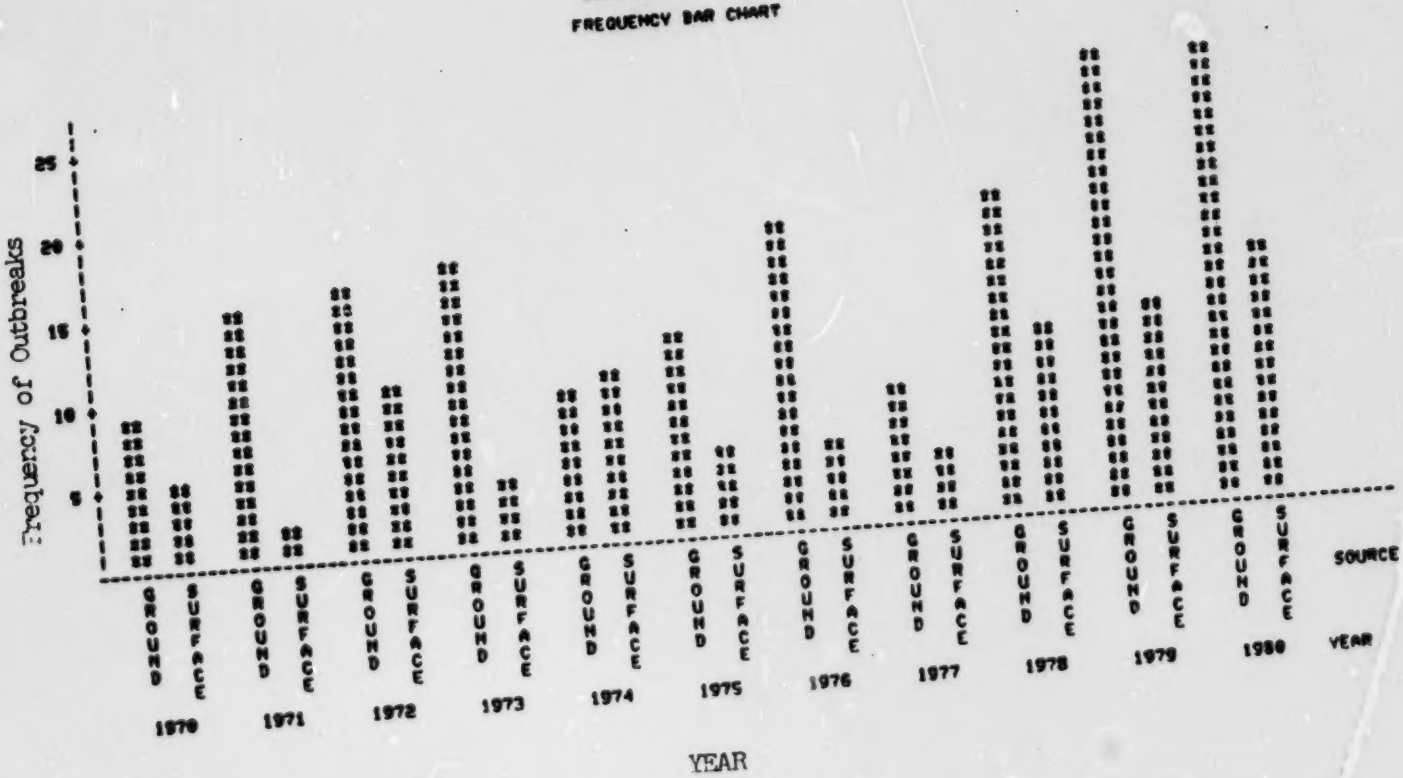
Source: Lippy and Waltrip, 1984

A comparison of the number of waterborne disease outbreaks due to ground and surface waters during the years 1970-1980 is provided in Figure I-3 (Lippy and Waltrip, unpublished data). With rare exceptions, outbreaks due to ground waters considerably exceeded those due to surface waters. The data in this figure have not been adjusted to compensate for the materially greater usage of ground waters as supply sources (see Figure I-4). A somewhat similar pattern has been found upon comparing community and non-community water systems, in which the non-community systems were responsible for more disease outbreaks (but fewer cases) than the more frequently monitored community water systems (Figure I-5) (Lippy and Waltrip, 1984). These data indicate that ground water surveillance must not be ignored, especially in non-community systems.

Purpose and Scope of Document

Total coliform bacteria have been used for more than 60 years for assessing the quality of drinking water (Evans *et al.*, 1981b). Other organisms have also been proposed to replace or complement this group. Some will be identified in Chapter III. This document will review the data to evaluate whether total coliforms should continue to serve as an indicator. It will examine this group's strengths and limitations and compare them to other candidate indicator systems. Other issues relating to total coliforms pertinent to standard setting will also be examined.

Fig. I-3

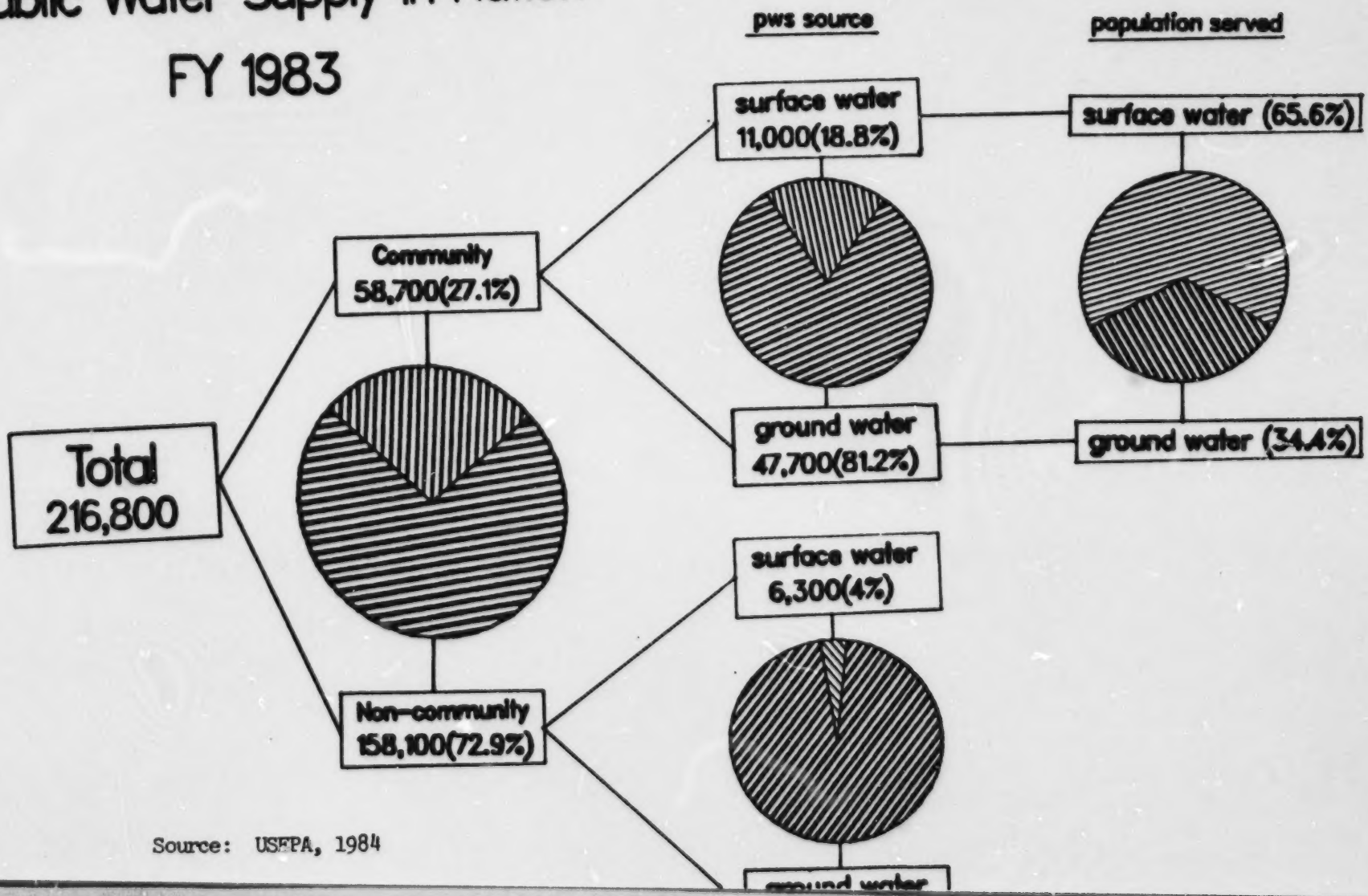


Source: Lippy, unpublished

Fig. I-4

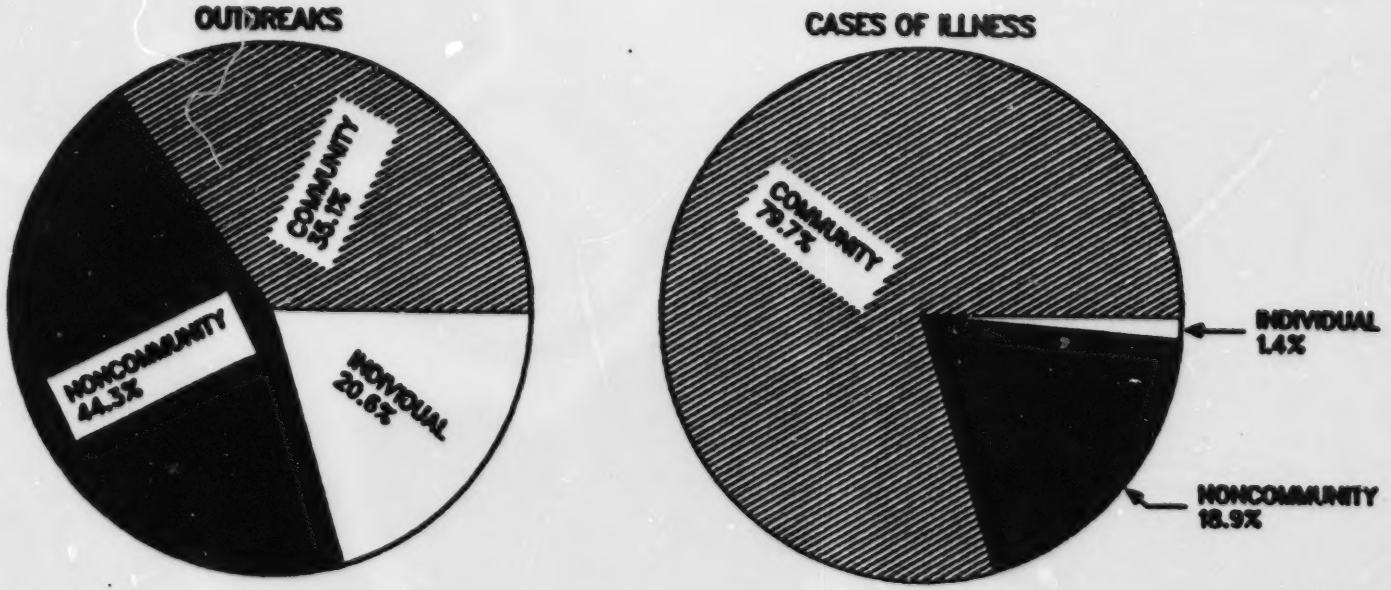
Public Water Supply in Nation

FY 1983



Source: USEPA, 1984

Fig. I-5. WATERBORNE OUTBREAKS/CASES OF ILLNESS, 1946–1980
BY TYPE OF SYSTEM



More specifically, Chapter II will identify the pathogens often found in drinking water, indicate their significance, and signify the utility of total coliforms in predicting their potential presence. Chapter III reviews the characteristics of an ideal indicator and assesses how well total coliforms and other candidate indicator organisms conform to the ideal. Chapters IV and V discuss transit time, methodology issues, coliform monitoring, and chlorine substitution. Chapter VI considers risk analysis, and Chapter VII presents tentative conclusions and recommendations.

This document occasionally refers to recommendations from a panel of experts at a microbiology workshop sponsored by USEPA's Office of Drinking Water (ODW), in conjunction with the American Society for Microbiology, in December 1981. The purpose was to examine options on revising the current drinking water microbiology and turbidity standards, and make recommendations to ODW. Internationally-recognized drinking water experts were assigned to one of six panels, each charged with different topics for discussion. The topics were: (1) Agents of Waterborne Disease, (2) Measurement of Microbial Water Quality, (3) Monitoring, (4) Methods, (5) Source, Treatment and Distribution, and (6) Compliance and Policy Issues. The workshop proceedings were published (Berger and Argaman, 1983).

II. WATERBORNE DISEASE AGENTS

General

Most of the organisms in water are not known to pose a hazard to healthy individuals. A few, however, may be primary or opportunistic pathogens. Some of these pathogens are associated with fecal matter (e.g., Salmonella, Shigella); others are free-living in the aquatic environment (e.g., Pseudomonas aeruginosa, Legionella). Categories of microbial pathogens implicated in waterborne illness include protozoa, bacteria, and viruses. This Chapter will identify the pathogens which have been implicated in waterborne disease in the United States, and briefly indicate the utility of coliforms in detecting their presence.

Microbial agents implicated in waterborne disease in the United States between 1946 and 1980 are indicated in Table II-1 (Lippy and Waltrip, submitted for publication). The number of outbreaks and cases associated with each is also presented. In about half the outbreaks, the etiological agent was not identified. Since 1971, the only microbial agents implicated in waterborne disease have been Giardia, Salmonella, Shigella, enterotoxigenic E. coli, Yersinia enterocolitica, Campylobacter fetus, hepatitis A agent, Norwalk agent, and rotavirus (CDC, 1980-1983). Vibrio cholera was also implicated in a single outbreak with 17 cases, the largest cholera outbreak yet in the United States during the 20th century (CDC, 1982b). According to CDC, Yersinia was not

Table II-1

CAUSATIVE AGENTS
WATERBORNE OUTBREAKS, 1946-1980

	No. of Outbreaks	Cases of Illness
BACTERIAL		
Campylobacter	2	3,800
Pasteurella	2	6
Leptospira	1	9
E. coli	5	1,188
Shigella	61	13,089
Salmonella	75	18,590
	146	36,682
VIRAL		
Parvovirus-like	10	3,147
Hepatitis	68	2,262
Polio	1	16
	79	5,425
PARASITIC		
Entamoeba	6	79
Giardia	42	19,734
	48	19,813
CHEMICAL		
Inorganic	29	891
Organic	21	2,725
	50	3,616
UNKNOWN	350	84,939
TOTAL	672	150,475

(Source: Lippy and Waltrip, 1984)

implicated in any waterborne disease between 1946-1980 (Lippy and Waltrip, 1984), but has been in at least 2 incidents since 1980 (CDC, 1982c; CDC, 1983).

An expanded list of agents which have either caused or could potentially cause a waterborne disease outbreach is found in Table II-2, along with some basic information regarding each agent. The range of times between infection and onset of clinical symptoms for selected agents is presented in Figure II-1. From the Table and Figure, it is apparent that the infective dose for waterborne bacterial pathogens is high, but onset of symptoms is rapid, while the reverse is true for ingested protozoan pathogens.

Waterborne Pathogens and Predictive Value of Coliforms

Sobsey and Olson (1983) compiled from the literature the survival times of waterborne pathogens and coliforms in natural and finished water. They reported that the survival of Salmonella, Shigella, and Campylobacter is generally similar to that of indicator bacteria such as E. coli. The authors also reviewed the literature on waterborne Yersinia enterocolitica, and concluded that this organism is poorly correlated with total or fecal coliform levels.

In addition to primary pathogens, a variety of bacterial opportunistic pathogens are common in finished water supplies (LeChevallier et al., 1980; Olson and Hanami, 1981). Many are

Table II-2. Summary of Taxonomic, Clinical and Epidemiological Features of Potential Drinking Water Disease Agents

Name of Organism or Group	No. Types	Major Disease	Major Reservoirs/ Primary Sources	Conc. in Primary Source	Infect. Dose	Prevalence (Avg. % Excretion)	Duration Of Shedding	Carrier State*
Bacteria								
Salmonella typhi	1	typhoid fever	human feces	10^6/g	low	[<0.1]	[typically 4 weeks, occ. >1 year]	•
Salmonella paratyphi	1	paratyphoid fever	human feces	10^6/g	high			
Other salmonella	>2,000	salmonellosis	human/animal feces	10^6/g	high			
Shigella	4	bacillary dysentery	human feces	10^6/g	high			
Vibrio cholerae	?	cholera	human feces	10^6/g	medium		<1 week	•(?)
Enteropathogenic E. coli	?	gastroenteritis	human feces	10^6/g	high		?	?
Yersinia enterocolitica	?	gastroenteritis	human/animal feces	?	high	1.2-15.5	?	•
Campylobacter jejuni	>1,000	gastroenteritis	human/animal (?) feces	?	?	?	?	?
Legionella pneumophila and related bacteria	?	acute respiratory illness (legionellosis)	thermally enriched waters	?	high?	?	1-3 weeks	?
Mycobacterium tuberculosis	1	tuberculosis	human resp. exudates	?	?	?	?	?
Other (atypical) mycobacteria	≥2	pulmonary illness	soil and water	?	?	?	?	?
Opportunistic bacteria	?	variable	natural waters	?	?	?	?	?
Enteric Viruses								
Enteroviruses								
Polioviruses	3	poliomyelitis	human feces					
Coxsackieviruses A	23	aseptic meningitis	human feces					
Coxsackieviruses B	6	aseptic meningitis	human feces					
Echoviruses	31	aseptic meningitis	human feces	10^6/g		[<1-5%]	1-3 weeks	
Other enteroviruses	>4	aseptic meningitis	human feces					
Reoviruses	3	AHC, encephalitis	human feces					
Rotaviruses	>3	mild UR and GI ill.	human/animal feces	10_2 part/g	low	?	1-2 weeks	
Adenoviruses	37	gastroenteritis	human feces			?	1 week	
Hepatitis A virus	1	UR and GI illness	human feces	10^6/g		?	?	?
Norwalk and related GI viruses	>3	infectious hepatitis	human feces			?	>3 weeks	
		gastroenteritis	human feces			?	≤1 week?	
Protozoans								
Acanthamoeba castellani	1							
Balantidium coli	1	amebic meningoencephalitis	soil and water	?	?	very low	none	•
Entamoeba histolytica		balantidiasis (dysentery)	human feces	?	?	very low	variable	?
Giardia lamblia	1	amebic dysentery	human feces	?	?	very low	variable	•
Naegleria fowleri		giardiasis (gastroenteritis)	human & animal feces	10^2/g	low	3-10	6-7 weeks	•(months to years)
		primary amebic meningoencephalitis	soil and water	10^3/g	low	1.5-2.0	none	
Helminths				?	?	very low		
Nematodes (roundworms)								
Ascaris lumbricoides	1	ascariasis	human (& animal) feces	$10-10^6$/g		<1		
Trichuris trichiura	1	trichuriasis	human feces	$10-10^6$/g		<1		•
Hookworms								
Ancylostoma duodenale	1	hookworm disease	human feces	$10-10^6$/g	low	<1	[Usually months, occas. years]	•
Necator americanus	1	hookworm disease	human feces	$10-10^6$/g		<1		•
Strongyloides stercoralis	1	threadworm disease	human & animal feces	$10-10^6$/g		<1		•
Algae (blue-green)								
Anabaena flos-aquae	6**+	gastroenteritis	natural waters	10^6-10^6	?	-	-	-
Microcystis aeruginosa	4+	gastroenteritis	natural waters	10^6-10^6	?	-	-	-
Aphanizomenon flos-aquae	1+	gastroenteritis	natural waters	10^6-10^6	?	-	-	-
Schizothrix calcicola	1	gastroenteritis	natural waters	10^6-10^6	?	-	-	-

*Untreated subclinical and/or asymptomatic infections.
**Numbers refer to number of different toxic strains.
+Also produces neurotoxins whose role in human disease is uncertain.

Source: Sobsey and Olson, 1983

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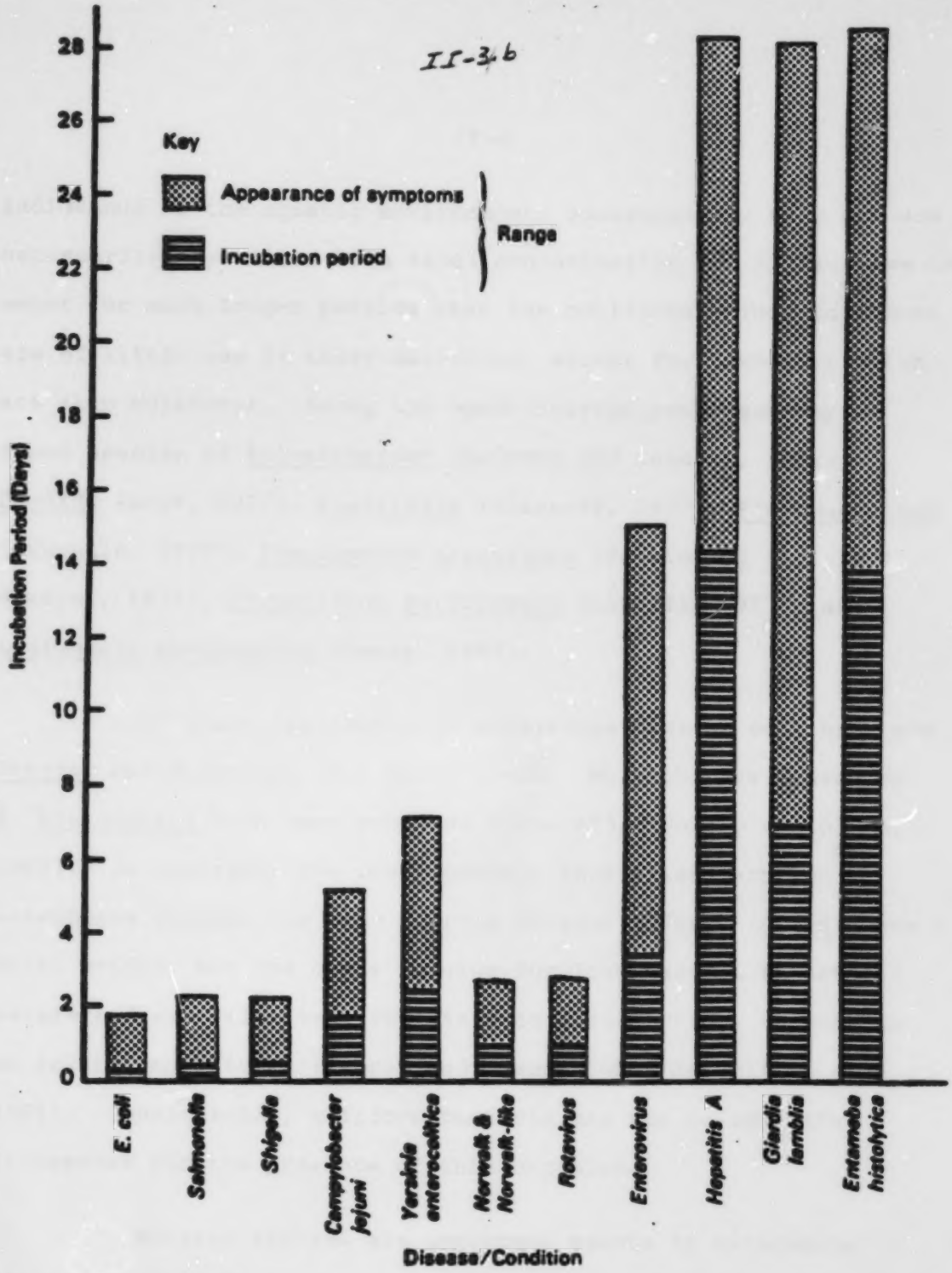

Fig. II-1. Comparison of Incubation Periods and Appearance of Symptoms in Waterborne Disease.

Source: adapted from CDC, 1982b

indigenous to the aquatic environment; consequently, they are not necessarily associated with fecal contamination and may survive in water for much longer periods than the coliforms. Thus coliforms are of little use in their detection, except for pathogens which are also coliforms. Among the opportunistic pathogens may be found species of Acinetobacter (LaCroix and Cabelli, 1982), Candida (Buck, 1977), Klebsiella (Vlassoff, 1977), Flavobacterium (duMoulin, 1979), Pseudomonas aeruginosa (Favero, et al., 1971; Hoadley, 1977), Clostridium perfringens (Cabelli, 1977), and Legionella pneumophila (Beaty, 1984).

Protozoa implicated in waterborne disease outbreaks are Giardia and Entamoeba (see Table II-1). No outbreaks caused by E. histolytica have been reported since 1953 (Sobsey and Olson, 1983). In contrast, the most commonly identified pathogen of waterborne disease has been Giardia (Craun, 1978b). Giardia has a fecal origin, but the cysts survive for long periods in natural waters and are relatively resistant to disinfection, especially at lower temperatures (Sobsey and Olson, 1983; Jarroll et al., 1981). Consequently, coliform bacteria may not be adequate indicators for the presence of this organism.

Enteric viruses are important agents in waterborne disease and usually enter water supplies through contamination with sewage (WHO, 1979). Viruses implicated in recent waterborne

outbreaks include the Norwalk agent, rotavirus, and hepatitis A (CDC, 1980-1983). Since viruses are obligate parasites, they are unable to multiply in water but may remain viable for extended periods of time (AWWA, 1979). Most enteric virus disinfection data indicate that they are more resistant than indicator bacteria (AWWA, 1979; Sobsey and Olson, 1983). This suggests that coliforms are not an adequate indicator for viral pathogens.

Blue-green algae (cyanobacteria) have been associated with several waterborne disease outbreaks in the U.S. (Billings, 1981; Skykora and Keleti, 1981), but none have been conclusively implicated in these outbreaks. Symptoms can include gastrointestinal distress, respiratory distress, sore throats, and other effects (Billings, 1981). Coliforms are inadequate indicators for blue-green algal toxins (Sobsey and Olson, 1983).

Quantitative Relationship between Coliforms and Pathogens

Studies attempting to relate coliform density and pathogen density in water are sparse. Some involve ambient water, others involve estuarine water and raw and treated sewage (Geldreich, 1978; Vaughn et al., 1983; Dutka, 1973). No publications were found pertaining to treated drinking water. In most of these reports, coliform and either Salmonella or enterovirus density ratios were determined, undoubtedly because, unlike the

case for most other pathogens, reasonably adequate recovery and analytical procedures exist for these two groups of pathogens. Because ratios in ambient water are more relevant for predicting associations in drinking water than ratios in estuarine water or sewage, only ambient water data are considered in this section.

An early investigation by Kehr and Butterfield (1943) attempted to relate coliform densities to typhoid fever incidence. As part of this effort, they examined the density of Salmonella typhi per 10^6 total coliforms in several previously published studies. The data generally show ratios of 1-100 S. typi per 10^6 coliforms for polluted river water and sewage.

Dutka (1973) compared salmonellae densities with the densities of total coliforms, fecal coliforms, and fecal streptococci in the relatively unpolluted St. Lawrence River. For five observations, the total coliform/salmonellae ratios were 66,000:1, 42,000:1, 7,000:1, 4,900:1, and 650:1. Salmonellae were recovered from water containing as few as 7 coliforms/100 ml. Ratios for fecal coliforms ranged from 95-16,000:1 and for fecal streptococci were 95-1,500:1. Based upon this study, a total coliform/salmonellae ratio cannot be defined.

In an investigation prompted by a waterborne outbreak in Riverside, California, which affected 18,000 people, researchers detected the etiologic agent, Salmonella typhimurium, in source water and in the distribution system (Ross et al., 1966; Boring et al., 1971). The source water (from deep wells) was not treated before entry into the distribution system. The densities for E.

coli and S. typhimurium in a composite water sample were 1.4/liter and 17/liter, respectively (Boring et al., 1971). Thus for this outbreak situation, the E. coli/S typhimurium ratio was 0.07. Total coliform data were not provided in the Boring et al. (1971) publication.

Stetler et al. (1984) compared the concentration of viruses and several indicator organisms in the source and treated waters of a conventional water treatment plant in Michigan. The source water was a small river containing secondary- or tertiary-treated sewage effluents from several small towns. Total coliform: enterovirus ratios (CFU/100 ml: PFU/380 liters) varied from 1:1 to over 46,000:1 for eleven samples. The investigators reported that on days when the highest virus recovery occurred, the concentration of total coliforms and other bacterial indicators were almost always low to moderate; conversely, when total coliform concentrations were highest, virus recovery was lowest. Thus, total coliform concentrations in this study did not correlate with virus densities.

Berg and Metcalf (1978) examined concurrent occurrence data on enteroviruses and fecal coliforms in the Missouri and Mississippi Rivers. Variations in the fecal coliform:virus ratio (CFU/100 ml:PFU/100 ml) were marked. Ratios ranged from 14,000:1 to 7,600,000:1 for 6 Missouri River water samples and 1,000:1 to 7,500,000:1 for 12 Mississippi River water samples. In 4 of these 18 samples, viruses were recovered from waters containing 400 or fewer fecal coliforms/100 ml. The variation in total

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coliform:enterovirus ratios was not examined in this study, but should be even more pronounced than with fecal coliforms.

In another investigation, total coliforms were not detected in the finished water of a conventional water treatment facility in the presence of polioviruses, with densities in finished water as high as 1 cytopathogenic unit/liter (Payment, 1981). Densities generally ranged, however, from 1-10 units/100 liters. The source water was sewage-contaminated river water. It is noteworthy that the human ID_{50}, ID_{10}, and ID_1 for poliovirus type 1 vaccine administered orally are 72, 39, and 20 tissue cultured doses, respectively (Minor et al., 1981). For some enteroviruses, a single infectious unit is capable of inducing infection in humans (WHO, 1979).

Vaughn et al. (1978) compared enterovirus densities with coliform levels in wastewater-recharged groundwater. Three sites were used. In each, chlorinated effluents were discharged into basins, which were 30 feet, 80 feet, or 18 feet above groundwater. Wells were constructed which were 10 feet, 8 feet, or 150 feet down gradient from the basins. Thirty observations of well water were reported. In 23 of the 30 observations, no viruses were detected. In 6 cases where viruses were found, densities and ratios (total coliforms/100 ml:PFU/gallon) were 23,000:1.3, 23:3.6, 15:2.4, 430:3.7, 430:1.6, and 15:10.6. It is apparent that the total coliform/virus ratios range over 4 orders of magnitude in this study. Calculations based on these data also suggest that source waters may

have virus densities less than two orders of magnitude lower than total coliforms.

Several studies provide total coliform densities and merely indicate whether specific pathogens were detected, rather than attempting to determine a density ratio. Gallagher and Spino (1968), for example, examined data from five previous studies of this type. In the 16 observations of river water reported, salmonellae were detected in 10 instances. For these 10 cases, total coliform density means ranged from 682-12,000/100 ml. Where salmonellae were not detected, total coliform means ranged from 300-16,000/100 ml. Since there were no observations between a total coliform level of 300 and 682/100 ml, the data do not demostrate a total coliform density below which Salmonella bacteria cannot be detected.

Seligmann and Reitler (1965) examined the relationship between E. coli levels and detection of Salmonella in water (mostly from springs) tentatively implicated in sporadic cases of enteritis. In 10 observations where Salmonella (or Arizona in one instance) were detected, E. coli densities per 100 ml ranged from 0-130,000. In six of these, E. coli counts were 20 or less. Total coliform data were not collected.

In summary, high levels of total coliforms are normally present in ambient water, but their numerical relationships with specific coexisting pathogens are extremely variable. Salmonella has been found in ambient water containing as few as 7 total coli-

forms/100 ml, while 10.6 viral PFU/gallon was found at 15 total coliforms/100 ml. Data on the quantitative relationships between total coliforms and *Shigella* and other waterborne pathogens are apparently lacking.

III. USE OF TOTAL COLIFORMS AS INDICATORS

Necessity for Indicators

The primary reason for evaluating the microbial quality of drinking water is to prevent waterborne disease outbreaks. More specifically, monitoring is accomplished to assess treatment efficiency, to detect deterioration of water quality in the distribution system, and to signal the potential presence of fecal contamination.

Ideally, specific detection of the various organisms causing waterborne disease would be the most direct approach in reducing their hazards. Unfortunately, in actual practice, this is quite impractical because of a number of limitations. There is a large variety of potential waterborne pathogens, including bacteria, viruses, and protozoa, and a search for all would be extremely difficult. Moreover, if pathogens are present, their concentrations in water would usually be sufficiently small so as to require large-volume samples. Many are so fastidious in their environmental and nutritional requirements that only highly specialized laboratory techniques can be used to detect them, if they can be detected at all. Some cannot yet be cultivated in the laboratory, or barely so, and efficiency of water recovery and detection can be very low. Finally, extended delays would be involved in carrying out identification procedures, even with the extremely doubtful assumption that every laboratory involved

in water testing would have, or could afford, the qualified personnel and other resources to accomplish this.

Decades ago, it became apparent that a more reasonable and practical method for signalling the presence of fecal contamination and treatment efficiency was required. The indicator concept was derived from this line of reasoning. The indicator was defined as a single organism (or chemical), or small group of organisms, which were easily detectable and which could signal the presence of fecal contamination or at least provide reasonable assurance that treatment was adequate to minimize waterborne disease outbreaks.

Characteristics of an Ideal Indicator System

Characteristics of an ideal indicator have been listed by several authors (Bonde, 1963, 1977; NAS, 1977), and they generally include the following:

1) They should be applicable to all types of water.

2) They should always be present in sewage and fecally polluted waters when pathogens are present.

3) Their numbers should represent the degree of pollution.

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4) They should be present in much greater numbers than the pathogens and therefore still detectable even after great dilution.

5) They should not reproduce in water, or at least, not more than the pathogens.

6) They should have a longer survival time than the pathogens in the aquatic environment and in the presence of disinfection and other treatment.

7) They should not be found in unpolluted waters.

8) They should be detectable by simple, inexpensive laboratory tests in a short period of time.

9) They should have constant characteristics.

10) They should be non-pathogenic to man and animals.

Comparison of Candidate Indicator Systems With the Ideal Indicator

Total coliforms and fecal coliforms are the most commonly used microbial indicator systems for monitoring drinking water quality (NATO, in press). Various other organisms or groups of organisms have been proposed. In the following sections, several of these candidates are compared with the attributes of the "ideal" indicator system. Subsequently, the most important candidates are compared to each other.

Total Coliforms

The total coliform group is defined in Standard Methods (APHA, 1980) as "all aerobic and facultative anaerobic, gram negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas-formation within 48 hrs at 35°C." Escherichia coli and Klebsiella (Vlassoff, 1977) would be included in this general group as well as Enterobacter, Citrobacter, and (arguably) Aeromonas (Ptak and Ginsburg, 1977; Seidler and Evans, 1983). Unfortunately, coliforms do not represent a homogeneous taxonomic group (NAS, 1977).

The total coliform method for determining the microbiological quality of drinking water has been the subject of considerable criticism. Among the criticisms are that coliforms are frequently not detected when in large volumes of water (Mack, 1977), and also may not be detected when in the presence of large numbers of interfering non-coliform bacteria (Geldreich et al., 1977; Herson and Victoreen, 1980). In addition, coliforms stressed in the aquatic environment by chlorine, heavy metal ions, antagonistic organisms, and other factors may grow poorly on standard coliform media, resulting in very substantial underestimates of their number (McFeters et al., 1982).

Total coliforms may be present in all types of waters (Kott, 1977). Generally, they are detectable when enteric pathogenic bacteria are potentially present, although outbreaks of disease due to Salmonella, Shigella, and other agents have occurred

in waters in which total coliforms were not found (Hejkal et al., 1979; Payment, 1981; Craun, 1978a).

Generally, the greater the number of total coliforms, the greater the degree of pollution. In addition, coliforms far outnumber enteric pathogenic bacteria and are generally detectable even after great dilution.

The ideal indicator should not reproduce in water. Some of the coliform species do, but this would not significantly affect the interpretation of the sanitary quality of drinking water since errors would be on the side of safety. In one sense, this problem is not of great consequence since the primary function of total coliforms is to measure treatment efficiency.

Considerable evidence has been provided that total coliforms and a number of the enteric bacterial pathogens have roughly similar survival times in water as well as sensitivity to disinfection (McFeters, 1974; Wang et al., 1983; Sobsey and Olson, 1983). This is not true, however, for Giardia and some of the other more resistant pathogens. Thus total coliforms do not entirely satisfy this criterion for an ideal indicator.

One criticism of the coliform group as indicators is that their presence in water does not necessarily indicate fecal pollution. Some members of the coliform group are ubiquitous in nature (Geldreich et al., 1964; Stuart et al., 1971; Stuart

et al., 1976) and McFeters et al. (1978) even found "natural" coliform bacteria in a pristine mountain stream. Consequently, some members of the total coliforms are not associated with fecal contamination and may proliferate readily in a dilute aquatic environment (Dufour, 1977; Sjogren and Gibson, 1981). While total coliforms do not meet this criterion for an ideal indicator (item 5), this does not affect their utility as a gauge of treatment efficiency (or regrowth problems in the distribution system).

The test for total coliforms complies readily with the requirement that indicator tests should be relatively simple and inexpensive. A minimum of 22-48 hours of incubation is required with conventional tests before results can be determined (APHA, 1980).

The ideal indicator organism should have constant characteristics which would permit easy and infallible recognition. This is not totally realized with the coliforms (NAS, 1977). The ability to ferment lactose with the production of gas may be lost. The production of a metallic sheen by some coliforms may also be lost, inhibited, or obscured (Evans, et al., 1981c). Fortunately, in most cases, enough of the original characteristics are retained to provide positive tests.

The criterion of non-pathogenicity is not strictly satisfied by total coliforms. The existence of enteropathogenic E. coli is well-known and strains of Klebsiella are opportunistic

pathogens (Vlassoff, 1977). In general, however, most strains of these organisms do not appear to be pathogenic.

Fecal Coliforms and E. coli

The fecal coliforms may be defined as coliforms capable of fermenting lactose with the production of acid and gas at 44.5°C within 48 hours (Geldreich, 1966). Dufour (1977) also adds much more restrictive requirements for defining fecal coliforms, which, in effect, defines fecal coliforms as E. coli: does not produce cytochrome oxidase, produces indole from tryptophane, cannot utilize sodium citrate as a sole source of carbon, cannot produce acetylmethylcarbinol, cannot hydrolize urea, and gives a positive methyl red test. While the fecal coliform test is often regarded as a test for E. coli, other organisms may be included, especially Klebsiella (Dufour, 1977). Unlike other coliform species which occur in diverse natural habitats, the gastrointestinal tract is the only normal habitat for E. coli (DuFour, 1977).

The fecal coliform test is applicable to many types of waters and is used in many European countries as the sanitary test for drinking water (Muller, 1977). In the United States, this test has been used in studies on stream and lake pollution and sewage effluents, raw water sources, bathing waters, and seawaters. Fecal coliform densities correlate more closely with fecal contamination

than total coliforms (Deaner and Kerri, 1969). They are always present in sewage and fecally polluted waters (Dufour, 1977) and when found, indicate fecal pollution and the possible presence of enteric pathogenic bacteria. Among the coliforms in human feces, 96.4% are fecal coliforms (Geldreich et al., 1962). The presence of E. coli is generally considered as a measurement of fresh fecal contamination (Bonde, 1977). It is not found in unpolluted waters. In special circumstances, it is more likely for water to contain pathogens in the absence of detectable fecal coliforms than in the case of total coliforms since some pathogens have greater survivability than the fecal coliforms (Boring et al., 1971; Seligmann and Reitler, 1965), and total coliforms outnumber fecal coliforms.

Fecal coliforms are more difficult to detect in water after great dilution than the total coliform group since fecal coliforms are far less numerous in contaminated waters than this group and therefore make the test less sensitive (NAS, 1977). Fecal coliforms rarely reproduce in water, except where the nutrient load is high (Geldreich, 1978; McFeters et al., 1978). According to Geldreich (1978), an analysis of data from numerous stream pollution investigations indicate that fecal coliforms will not persist in waters with a BOD of less than 30 mg/l. McFeters et al. (1978) found that E. coli numbers increased by an order of magnitude when inoculated into sterile extracellular

products of the alga Chlorella. The minor component of the fecal coliform group, Klebsiella, is ubiquitous in nature and is capable of regrowth in ambient waters receiving nutrient rich (high BOD) wastes from paper mills, textile mills, and potato processing plants (Bagley and Seidler, 1977).

Fecal coliforms are readily detectable employing Standard Methods procedures. Like total coliforms, some strains of E. coli may produce atypical colonies on MF media or be anaerogenic using the fermentation tube method (Evans, et al., 1981c). Consequently, their characteristics are not always constant. A few strains of E. coli and Klebsiella are opportunistic pathogens (Vlassoff, 1977; Greeson, 1981).

Fecal Streptococci/Enterococci

The fecal streptococci are found in the feces of human and warm-blooded animals and may be recovered from polluted waters. Their presence in water is generally considered to be evidence of fecal pollution (Clausen et al., 1977), although some species are capable of reproduction external to warm-blooded animals (Mundt, 1963a,b). These organisms appear to survive longer in water than many fecal pathogens and fecal non-pathogens (McFeters et al., 1974; Miescier and Cabelli, 1982). Fecal streptococci may have some utility in indicating the potential presence of viral contamination (Clausen et al., 1977).

Streptococcus species which can be recovered from feces in significant quantities are referred to as fecal streptococci. The enterococci are species of fecal streptococci which conform to certain growth criteria (e.g., ability to grow at both 10°C and 45°C). The term "enterococcus" generally refers to two species, *S. faecalis* and *S. faecium* (APHA, 1980).

Two species of the fecal streptococci, *S. bovis* and *S. equinus*, are normally found only in the feces of nonhuman, warm-blooded animals and are useful in identifying pollution originating from feedlot runoff, dairy plant wastes, and discharges from meat and poultry processing operations (Geldreich, 1978). These two species do not survive for long outside of the animal intestinal tract, and their presence is an indication of recent pollution (Geldreich, 1978). Some of the fecal streptococcus group and enterococcus group have limited sanitary significance (Clausen *et al.*, 1977).

Fecal streptococci have been found in various types of waters (Miescier and Cabelli, 1982; McFeters *et al.*, 1974) and show good correlation with waterborne disease in estuarine environments (Cabelli *et al.*, 1982). Additional work appears to be needed to establish relationships between their numbers and the degree of pollution. They appear to be present in greater numbers than many waterborne pathogens and are readily detectable after great dilution (NATO, in press; Miescier and Cabelli, 1982).

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According to Geldreich (1978), S. faecalis var. liquifaciens, an enterococcus, is ubiquitous in nature and generally predominates at counts below 1 fecal streptococcus per ml. Thus use of fecal streptococci in clean water may be unreliable (Bonde, 1977).

The fecal streptococci are readily identified and speciated by ordinary laboratory methods (APHA, 1980), although recovery rates and poor agreement among various methods is a problem (NAS, 1977). They are not considered to be pathogenic (Clausen et al., 1977).

Clostridium perfringens

Of the more than 60 species of Clostridium, C. perfringens is the one most consistently associated with human fecal wastes (Cabelli, 1977). It is consistently present in human feces at a relatively high density (Bisson and Cabelli, 1980). There is controversy whether other important reservoirs exist, since C. perfringens spores are widely found in terrestial and aquatic environments (Cabelli, 1977). C. perfringens might be useful in determining the age of pollution occurrences since it has been found that the ratio of spores to vegetative cells increases with the distance from the source of pollution (Bonde, 1963). Moreover, E. coli:C. perfringens ratios have been proposed to elucidate the proximity of fecal pollution sources (Bisson and Cabelli, 1980). The survivability of Clostridium perfringens spores in water and their resistance to treatment compared to the pathogens is much

greater than other indicators (Bonde, 1977), perhaps excessively so (Olivieri, 1983). It appears that this organism is excreted in greater numbers than are fecal pathogens (NATO, in press).

C. perfringens can be identified rapidly and unambiguously (Bonde, 1977). The organism is an anaerabe, however, and the cost of culturing this organism under strict anaerobic conditions reduces its potential as an indicator. While creating anaerobiosis may be a disadvantage, this environmental condition has the advantage of restricting the environment in which other organisms can reproduce, a desirable feature of an indicator. C. perfringens is the major cause of wound infections and gas gangrene, but there is no evidence that waterborne transmission is significant in the epidemiology of these diseases (Cabelli, 1977).

Heterotrophic Plate Count

The Heterotrophic Plate Count (SPC) is frequently referred to as "standard plate count," "colony count," "total viable count," "total count," "plate count," "total bacterial count," "bacterial count," or "water plate count." These terms are misnomers implying that the technique can provide all the growth requirements of all bacterial cells in a water sample and yield an accurate enumeration of all living organisms. In reality, the plating method permits the growth of only a relatively modest

percentage of all heterotrophic microorganisms in a sample (Geldreich et al., 1972; Taylor et al., 1983.)

Heterotrophic bacteria are indigenous to all types of water. They are, of course, present in sewage and polluted waters in far greater numbers than the pathogens. Their numbers do not necessarily represent the degree of fecal pollution. Detection and enumeration methods are simple and inexpensive. The number of organisms present in a water sample may provide an estimate of its sanitary quality and signal problems in treatment efficiency and degradation of quality in the distribution system. In a recent study, 30% of the SPC types were found to be opportunistic pathogens (LeChevallier et al., 1980).

Miscellaneous Alternative Indicators

Other organisms have been mentioned in the literature as potential indicators of fecal contamination and treatment efficiency. These include Aeromonas (NATO, in press), Candida albicans (Buck, 1977), Bifidobacterium (Levin, 1977), Vibrio (Colwell and Kaper, 1977), Staphylococcus aureus (Evans, 1977), Pseudomonas aeruginosa (Hoadley, 1977), Klebsiella (Vlassoff, 1977), coliphage (NATO, in press), vaccine polioviruses (NATO, in press). Several chemicals have also been proposed (NATO, in press).

Comparison of Alternatives

As reflected earlier in this document, the ideal drinking water indicator should be able to signal the presence of fecal contamination, indicate efficiency of treatment, and disclose deterioration in the distribution system. A panel of experts in drinking water microbiology recently compared proposed candidates for assessing the biological quality of drinking water in terms of these three main criteria (Olivieri, 1983). The ratings are reproduced in Table III-1. According to this panel, E. coli is the most suitable indicator for fecal pollution. For indicating efficiency of treatment, total coliforms, plate counts, and free chlorine residual are most appropriate. Deterioration in the distribution system is best detected by total coliforms, plate counts, and chlorine residual. The most suitable overall indicator, based on the total number of "pluses," is the total coliform group.

According to the panel, the total coliform group is not as suitable for measuring fecal pollution as are E. coli, fecal coliforms, and enterococci. This was undoubtedly based on the fact that total coliforms include organisms which are widespread in the environment and not often associated with fecal pollution. The other groups are more closely associated with fecal pollution, especially E. coli. Total coliforms, however, are relatively more resistant to chlorine and other stresses

than are fecal coliforms and E. coli (Kott, 1977). Moreover, the number of fecal coliforms is markedly lower in source waters than total coliforms, resulting in a less sensitive test. Analysis of fecal streptococci is problematical because of low recovery rates and poor agreement between various methods (NAS, 1977). Treatment which provides coliform-free water should also reduce pathogens to minimal levels.

Although there are well-recognized limitations to the utility of total coliforms for measuring drinking water quality, this group has been well accepted for many decades in the U.S. as the indicator of choice for this purpose. The panel of experts confirmed that the total coliform group is still the best indicator available. This panel, however, pointed out that the relationship of total coliforms to the incidence of waterborne disease is not well established. Even so, Craun (1978a) found that in a majority of cases, water samples collected during outbreaks between 1971-1975 showed high levels of either total or fecal coliforms. There is evidence that for estuarine environments, enterococci are more closely associated with gastrointestinal tract disorders than are total coliforms and other indicators (Cabelli et al., 1982); this relationship, however, has not been established for drinking water.

There are sound reasons for supplementing total coliforms with other indicators. Turbidity, for example, can be measured quickly and thus serves as a far more rapid, albeit less sensitive, check on water quality. Analysis of fecal coliforms or E. coli

Table III-1

Candidate Indicator Rating For Each Aspect of Surrogate Measurements in Drinking Water

Indicator	Fecal Contamination (a)	Treatment (b)	Degradation Deterioration Recontamination (c)	Comments
Total Coliform	++	++++	++++	(b) best available-deficiencies relative to viruses and Giardia.
Fecal Coliform	+++	++	++	
E. coli	++++	++	++	
Fecal streptococci	++	++	++	
Enterococci	+++	+++	++	
Coliphage	-	+	-	(b) may be useful as viral surrogate data are limited.
Mycobacteria-Yeast	-	+	-	(b) potentially useful for this application-data limited.
Plate Count	-	++++	++++	(b) same as (b) for total coliforms
C. perfringens	++	++	-	(a) far more resistant to disinfection than others (c) not useful for degradation problems.
Bifidobacterium	++	-	-	(a) useful only for extremely recent contamination.

continued. . .

Table III-1 (Continued)

Indicator	Fecal Contamination (a)	Treatment (b)	Degradation Deterioration Recontamination (c)	Comments
Aeromonas	-	-	++	(a) has many alternate sources
Pseudomonas	-	+	+	(b) has potential for growth on activated charcoal.
Fecal Sterols	++	-	-	(a) not amenable to continuous assay.
Optical Brightness	-	-	++	(c) recontamination indicator
ATP	-	-	-	(c) need more research
LAL	-	-	-	(c) data limited for this use.
Epifluorescence	-	+	-	(b) may be useful-limited data available.
Disinfectant Residual	-	++++ (Free chlorine)	++++ Free chlorine)	(c) use with total coliforms and plate counts, excellent for operational test, free chlorine best.
C^{14} Sugar	-	+	+	
Turbidity	-	+++	+++	(b) should be used in conjunction with chlorine residual measurement.

Olivieri, 1983 (as amended)

probably provides greater safety for untreated drinking water supplies than total coliforms. Plate counts include opportunistic pathogens, and concentrations may correlate with disease among the compromised population. Plate counts are also useful for signalling possible interference with total coliform analyses. Loss of free chlorine residual can provide a rapid indication of possible contamination in the distribution system.

Standards of Other Nations

The microbiological standards for drinking water in North Atlantic Treaty Organization countries (NATO, 1984), the World Health Organization (WHO, 1971), and the United States are all very similar. Nearly all countries recognize the significance of coliform organisms as indicators of water quality (WHO, 1971; Muller, 1977; Barrow, 1977). More countries rely upon tests for total coliforms than fecal (thermotolerant) coliforms (NATO, in press). Sampling frequency and sample storage time practices for the NATO countries are shown in Table III-2.

The European Communities (1980) has issued a directive indicating that the maximum admissible concentration (as opposed to "guide level") for total coliforms, fecal coliforms, and fecal streptococci is to be 0/100 ml by the membrane filter method and less than 1/100 ml by the multiple tube method. Sulphite-reducing clostridia must not be greater than 1/20 ml by the multiple tube

method. Water is to be monitored "at the point where it is made available to the user".

The World Health Organization issued guidelines in 1971 for drinking water quality (currently being revised), and the primary bacterial indicator for this purpose is the total coliform group. *E. coli* is also to be monitored. Standards are more stringent for piped supplies than for unpiped supplies (WHO, 1971).

Japan (1982) and the USSR (Stofen, 1973) monitor for total coliforms and total counts. The Australians monitor for total coliforms and *E. coli* (Australia, 1980).

Table III-2 - Sampling and Sampling Storage, Drinking Water Analysis

	Canada	Denmark	FRG	France	Greece	Israel[2]	Italy
1. Frequency of required sampling							
a. Raw water	As required by control agency[1]	As required[1]	Voluntarily by producer[1]	As required[1]	Not specified[1]	As required[1]	—
b. Treated water	See c.	See c.	See c.	See c.	Not specified[1]	As required[1]	—
c. Distribution system	At least 4x1/month, depending on population served (Table and figure available)	At least 1x/year, depending on population served	Undisinfected 1/30,000m^3 distributed; disinfected: 1/15,000m^3 distributed	At least 3x/year; up to 1x/day for large supplies	At least 1x/depending on population served (Table available)	As required	—
d. Other	—	—	—	—	—	—	—
2. Sample size, ml	$\geq$ 100	$\geq$ 500	about 250[1]	$\geq$ 350	200	100, 200	200
3. Maximum storage time, hours	24	24	3, if refrigerated, 24-30 h	8	12	3, 0.5	6
4. Storage temperature, °C	Refrigerate if stored > 24 hrs	0-5	about 4	4-6	about 4	ambient, 4-10	4

Table III-2 - Sampling and Sampling Storage, Drinking Water Analysis (Continued)

	Netherlands	Norway	Spain	Sweden	UK	US
1. Frequency of required sampling						
a. Raw water	1x/4 weeks	As required	Large systems 1x/day; small systems 1x/month	Groundwater 1x/year; surface water 4x/yr	Large systems (> 10,000) 1-2x/week	Not required
b. Treated water	Groundwater 1x/week; surface water 1x/day	Large systems (> 10,000) 3x/week; medium systems (5,000-10,000) 2x/wk; small systems (1,000-5,000) 1x/wk, (<1,000) 1x/month	See a.	Large system (> 4,000) 1x/wk; small systems 1x/month	Large systems 5x/wk; small systems 1x/wk at least	Not required
c. Distribution system	1x/week	Same as for treated water	See a.	Not required	At least 1x/wk, depending on population served (Table available)	At least 1x/3 months, depending on population served (Table available)
d. Other	—	—	—	—	—	—
2. Sample size, ml	250-500	2 x 250	200-300	800	228	MF[3] 100; MPN[4] 5 x 10 or 5 x 100
3. Maximum storage time, hours	24	3, if refrigerated 30	8	4 h, if refrigerated at < 10°C, 36 h	6	30
4. Storage temperature, °C	0-4	2-10	2-4	< 10	4	ambient, 0-10

[1]Not legally specified.
[2]Israel reported results for Public Health Lab., Tel Aviv and National Water Co.
[3]MF = Membrane filter procedure.
[4]MPN = Most probable number or multiple tube dilation procedure.

Source: NATO (in press)

IV. COLLECTION AND ANALYTICAL PROCEDURES

Sample Collection And Storage

Providing the laboratory with a reasonably representative sample is extremely important. When a sample is taken, it is representative only of the water passing the sampling point at that place and at that time. It may not be representative of the whole system and must not be considered as such. Samples collected from a water distribution system should be randomized in regard to location and time as much as possible (Pipes and Christian, 1982), the rationale being to vary the time of collection and to include as many widely scattered outlets as practical.

The time elapsing between collection and analysis of water samples may have a pronounced effect upon the results obtained, particularly if the temperature of storage and time delays are not carefully controlled (Lonsane et al., 1967). It is important to analyze samples as soon as possible after collection (Standridge and Lesar, 1977; Pipes and Christian, 1982; and McDaniels and Bordner, 1983). McDaniels and Brodner (1983) found that in finished drinking water held at 5° and 22°C, coliform densities declined substantially after 24, 30, and 48 hours, but not at 2, 6, or 12 hours. They recommend that samples be iced and analyzed within 6 hours.

Standard Methods (APHA, 1980) states that if samples cannot be processed within one hour after collection, they should be

stored in an iced cooler during transit to the laboratory. The sample is to be analyzed within 30 hours of collection.

Pipes and Christian (1982) evaluated the possibility of contaiminating samples via several routes. These included contamination by (1) sampling in restrooms, (2) touching the water in the bottle, (3) carrying samples in ice-filled chests, and (4) carry-over during filtration.

With regard to sampling in public restrooms, paired samples were taken from 17 sites. One positive sample was obtained, and this was from a restroom faucet. The data suggest, but do not prove, the possibility of subsequent contamination from restroom faucets. The authors conclude that routine sampling should avoid public restroom facilities.

The potential for contamination by touching the water sample was examined by having 111 test subjects each place a finger into the mouth of a sterilized 125-ml sample bottle containing 100 ml of dechlorinated tap water. The bottle, with the finger in it, was then vigorously shaken for about 5 seconds and then capped, returned to the laboratory, and processed by the membrane filtration procedure. Despite this worse case situation, only 5.4% of the samples contained coliforms. Thus a positive coliform result should not necessarily be blamed on even a poorly trained collector.

The third potential way examined for contaminating a sample was transit in ice-filled chests. As the ice melts, the ice water may rise above the tops of the sample bottles, and be sucked into the bottles. The investigators immersed tightly-secured bottles filled with sterile phosphate buffer into ice water. After up to 4 hours, the buffer was analyzed by membrane filtration. They found that when the ice water contained $\leq$ 10 coliforms. Thus immersion in ice water could be a major route for contamination, unless it is devoid of coliforms.

The final route of contamination investigated was carry-over during membrane filtration. In this procedure, the same filtration unit is normally used for a number of samples. Thus, if one sample has a high concentration of coliforms, there is some possiblility that some coliforms which did not reach the filter membrane might be swept into subsequent samples. To minimize this occurrence, the prescribed technique calls for rinsing the sides of the funnel at least twice with 100 ml of sterile water. There are, however, various rinsing procedures. The investigators examined measuring sample volume in graduated cylinders vs. directly in the funnel, rinsing with a squeeze bottle vs. from a graduated cylinder, and using different brands of filter funnels. They found that there was little difference among the options, but they carry-over was a major concern. If a sample contains a large number of coliforms (>80/100 ml), subsequent contamination is highly probable. The investigators recommend ways of avoiding carry-over or recognizing it when it does occur.

In another study, Fujioka and Narikawa (1982) demonstrated that short exposure (15-30 minutes) of water samples to sunlight can stress indicators and prevent growth in 90% of water samples, while somewhat longer periods (1-2 hours) cause irreversible inactivation.

Analytical Procedures

Both the membrane filtration (MF) and most probable number (MPN) (often called multiple fermentation tube) techniques have long been widely accepted for enumeration of total coliforms in drinking water. Standard Methods (APHA, 1980) describes the protocol and the media used in the United States. Because of extensive coverage of total coliform methodology elsewhere (e.g., Seidler and Evans, 1983), this document will be limited to examining briefly the efficacy of the MF and MPN procedures in detecting and enumerating total coliforms and other selected topics.

Efficacy of MF Test

The MF technique is a more rapid and precise method for enumerating total coliforms than the MPN procedure (Thomas and Woodward, 1955; Clark et al., 1957). It tends to provide a population density about 22% lower than that of the five tube MPN procedure, primarily because the MPN overestimates the true density (Thomas and Woodward, 1955). The 23% bias compensates for statistical probabilities below the true sample density (Geldreich, 1981).

Unfortunately, several factors adversely affect the accuracy and usefulness of the MF test. The presence of high turbidity in the test water may prevent the use of the method since the pores in the filter become clogged. In addition, high-background, non-coliform bacterial counts in the sample also interfere since they may overgrow and obscure the coliforms. Dense growth results in confluency and prevents discrete colony formation (Clark et al., 1957).

Certain environmental factors may cause stress (injury) in indicator organisms, causing them to multiply slowly or not at all under standard conditions and may cause a water of poor quality to appear satisfactory. Environmental factors causing stress include extremes of temperature and pH, low concentrations of critical nutrients, toxic substances, storage, improper handling, and disinfectants (APHA, 1980; McFeters et al., 1982). McFeters et al. (1982) examined the recovery of five healthy and injured coliforms (four genera) from water on a variety of selective media used for the recovery of gram-negative bacteria. For m-Endo medium, recovery was 93% for healthy coliforms, but only 66% for injured coliforms. In comparison, recovery of injured coliforms by lactose broth and lauryl tryptose broth was 72% and 56% respectively. This group found this could be explained in part by the greater sensitivity of injured coliforms to high deoxycholate concentrations in the medium. Other researchers have suggested that stress may cause an underestimation of coliforms by up to 90 percent (Bissonnette et al., 1975; Dutka et al., 1979). This may also lead to difficulties in

recognition of sheen-producing colonies by the MF technique (Seidler and Evans, 1983).

Necessity for the MF Verification Test

A key factor in determining a positive or negative test by the MF technique is the ability to recognize colonies exhibiting a metallic sheen (golden-green) on an Endo-type medium. The appearance of such sheens at 24 hours is considered characteristic of members of the coliform group (APHA, 1980). Occasionally, there may be a doubtful appearance of sheen or no sheen at all from atypical coliform colonies. A number of additional factors may enter into mistaken identification of sheen production, such as poor sheen development due to sample turbidity, confluent growth due to heavy background bacterial content, water condensates, turbidity particles, and even confusion during analysis due to work under fluorescent lighting and a consequent mirror-like reflection. Any of these artifacts may create uncertainty with an inexperienced technician (Geldreich, 1981; Geldreich et al., 1967). In addition, synergistic false-positive reactions on Endo media may occur (Schiff et al., 1970).

The foregoing suggests the importance of verifying MF colonies. One verification test uses B-galactosidase and cytochrome oxidase assays (Pickett et al., 1981; LeChevallier et al., 1983). Another consists of transferring each suspected coliform colony to lactose broth (LB) or lauryl tryptose broth (LTB) for

evidence of gas production at 35°C within 48 hours. A gas-positive tube culture is confirmed in brilliant green lactose bile broth (BGLB) (APHA, 1975). BGLB can also be inoculated at the same time LB or LTB is inoculated.

A major difficulty with using LTB and BGLB in verification is that some coliforms may be anaerogenic in these media and yet aerogenic in other lactose-containing media (Evans et al., 1981c). Moreover, some coliforms may be anaerogenic in LTB, but produce gas in BGLB (Standridge and Delfino, 1982). Evans et al. (1981c) reported that at least 90% of the MF isolates aerogenic in LTB, LB, or m-LAC broth, but anaerogenic in BLBG broth were identified as coliforms. In contrast, Fifield and Schaufus (1958) found that verification by LB plus microscopic examination resulted in low percentages of false positives (7.7%) and false negatives (6.4%). To reduce suppression of gas production in lactose media, Evans et al. (1981c) proposed a new formulation, m-LAC broth, as a verification medium. According to their investigation, this medium identifies more coliforms colonies as coliforms than either LB or LTB.

Efficacy of MPN Test

The efficacy of the MPN test depends, among other factors, on the degree of environmental stress and disinfection injury of the coliforms, the media used, the number of coliform antagonists in the sample, and the ability of noncoliforms acting alone or

synergistically to produce gas from lactose. McFeters et al. (1982) have demonstrated that the number of chlorine-injured coliforms recovered on lactose broth (LB) and lauryl tryptose broth (LTB) are only 72% and 56%, respectively, of those recovered on nonselective media.

A variety of modifications for improving the MPN have been reported, most of them involving substitution of the culture media currently used, shortening the time required for the test, or increasing its sensitivity (Andrews and Presnell, 1972; Olson, 1978; Speidel, 1980; Evans et al., 1980; Evans et al., 1981a, b; Evans et al., 1981c; Seidler and Evans, 1982, 1983). These factors are major problems in the technique. Other disadvantages include extensive recordkeeping, considerable glassware requirements, and the number of steps involved in the technique (Standridge and Delfino, 1981). These are considered to be minor problems.

Although gas production in the Presumptive test indicates the probable presence of coliforms, there are a number of non-coliforms which acting alone, or synergistically, can produce gas from lactose and, therefore, give positive Presumptive tests. Most of the work on presumptive media evaluations was done during the period 1920-1930 and has been adequately reviewed by Seidler and Evans (1983). The Confirmed test would reduce these false positives.

The microbiology workshop in 1981, described in Chapter I, recommended a regulation to require that all turbid gas-negative

tubes in the Presumptive test be subjected to the Confirmed test to ensure that coliform masking has not occurred (Seidler and Evans, 1983). According to this group, the detection of coliform masking should dictate that 10 percent of all future turbid-only Presumptive tubes which were gas-negative but turbid (Geldreich et al., 1978; Olson, 1978; Evans et al., 1981b; Seidler et al., 1981). In one such case, Seidler et al. (1981) transferred cultures from turbid gas-negative Presumptive tubes (LB or LTB) into BGLB, EC Broth, and m-LES agar. A positive coliform reaction was produced in at least one of these three media for 20% of such transfers from LB and 36% from LTB.

Standard Methods (APHA, 1980) recommends Completed tests be performed on 10 percent of the positive Confirmed tests. Bowmer and Campbell (1972) found that only 61 percent of positive Confirmed tests gave positive completed tests; Geldreich et al. (1967) also found that the coliform densities of the MPN Completed test were significantly lower than the Confirmed MPN densities. It is not known whether the Completed test reduced the number of non-coliforms (false-positives) or coliforms found in the Confirmed test.

Microbiology workshop participants recommended that regulations not be established for conducting the Completed test because the medium employed, Eosin Methylene Blue, may be too inhibitory toward some coliforms (Seidler and Evans, 1983).

Interference With Total Coliform Analyses

Both the MF and MPN tests are subject to interference by high levels of heterotrophic bacteria. In the MPN test, the presence of coliforms is indicated by the production of gas in a lactose-based broth which collects in an inverted tube (Durham tube) within a larger test tube. There is evidence that some non-coliforms can produce gas in the Presumptive media either alone or synergistically with other organisms (Seidler and Evans, 1983). This causes a false-negative reaction, i.e., the production of gas when no coliforms are present. A more serious type of interference occurs when the presence of coliforms is masked by organisms which prevent their growth or sheen production because of the production of toxic substances, plate overgrowth, or by competitive inhibition. This phenomenon is termed a false-negative reaction.

Coliform antagonism has been demonstrated by such common aquatic bacteria as Pseudomonas, Micrococcus, Flavobacterium, Bacillus, Actinomyces, Acinetobacter, Arthobacter, Sarcina, and Moraxella (Hutchison et al., 1943; Means and Olson, 1981; Herson and Victoreen, 1980), and these comprised 35% of the SPC bacteria identified by LeChevallier et al. (1980) in chlorinated drinking water. Means and Olson (1981) found that 22.1% of 448 bacterial isolates from a chlorinated system produced bacteriocin-like substances inhibitory to total coliforms. In their investigation, Flavobacterium/Moraxella represents 57.1% of all bacteria producing

these toxic substances. There is also evidence that samples containing high SPC densities reduce coliform numbers during transit to laboratories (McDaniels and Bordner, 1983; Nash and Geldreich, 1981).

The adverse affect of high SPC populations on the MTF test for coliforms was indicated by an investigation in Karachi, Pakistan (Ahmed et al., 1964). In this study, enumeration of Salmonella, Shigella, SPC, and total coliforms in the potable water supply was performed. When the SPC exceeded 500/ml, the Salmonella and Shigella methodology became less sensitive (Table IV-1). These two pathogens were found in 22 of the 44 distribution samples which were negative for coliforms, and in 16 of these 22 (72.7%), SPC levels exceeded 500/ml. It appears that coliforms were suppressed even more than the two pathogens by the high SPC levels.

Seidler et al. (1981) also demonstrated that high SPC levels adversely affect the MFT method. In this study, samples of drinking water were collected from several communities which either disinfected only or practiced no treatment. Total coliform counts were assessed using the MFT technique in Standard Methods (both lactose broth and lauryl tryptose broth) and a more rigorous MFT procedure (M-MPN). Coliform counts from these MFT procedures were compared with the SPC densities in the samples. The data are shown in Table IV-2. As can be noted, coliform counts in all of

the MFT techniques used were reduced at SPC densities greater than 500/ml, especially when lauryl tryptose was used.

The same group examined private well and spring water supplies in a rural community of 78 households in Western Oregon (Lamka et al., 1980). Again they found an inverse correlation between SPC densities and coliform incidence, using the MFT technique (Table IV-3).

As mentioned previously, high-background, non-coliform organisms in water samples can also interfere with the MF procedure. SPC organisms can overgrow and obscure or prevent coliform colony formation or reduce sheen formation. To examine this phenomenon, Geldreich et al. (1978) collected 613 samples from flushes of dead-end water mains in Cincinnati. Analyses included MFT, MF, and SPC procedures (Table IV-4). The investigators found that 76 samples contained coliforms by the MFT procedure, but only 19 by the MF procedure. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the MF method. The investigators also surmised that SPC bacteria interfered with the MFT method, since the contents of tubes with heavy growth but no gas did produce gas when inoculated into brilliant green lactose bile broth. They concluded that the MF method, and probably the MFT method, is degraded when SPC densities exceed 500-1000 organisms/ml.

Table IV-1

Pathogen Occurrences in 125 Distribution Samples from Karachi Community Supply

Standard Plate Count per ml†	Number of Supplies	Salmonella-Shigella	
		Occurrence	Percent
<1	9	0	00.0
1-500	19	13	68.4
501-1,000	71	33	46.5
>1,000	26	11	42.3

*Selected data from Ahmed, Poshni and Siddiqui as reported by Geldreich et al. (1972).

†Pour plates incubated at 37C for 48 hours

Table IV-2

Impact of SPC Density in Drinking Water on Coliform Masking in the MPN Technique

SPC Density--colony forming units/mL	Number of Samples	Geometric Mean--Total Coliform Index 100 mL			
		Lauryl Tryptose Broth Presumptive Medium		Lactose Broth Presumptive Medium	
		S-MPN	M-MPN	S-MPN	M-MPN
<10	203	1.2	1.3	1.1	1.5
10 ≤ 49	19	1.4	2.5	1.4	2.9
50 ≤ 99	11	2.1	4.1	1.9	4.0
100 ≤ 249	9	2.7	11.0	2.8	6.5
250 ≤ 500	10	15.1	32.0	6.7	27.0
> 500	10	2.2	14.0	4.2	24.0

Seidler et al., 1981

Table IV-3

Standard Plate Count And The Incidence
Of Total And Fecal Coliform Detection

Standard plate count density range/ 1 ml	No. of samples	Total coliform occur-rences	Total coli-form inci-dence	Fecal coliform occur-rences
<10	61	1	1.6%	1
11-100	177	14	7.9%	3
101-500	52	4	7.7%	0
>500	19	1	5.2%	0

Source: Lamka et al., 1980

In another study, Geldreich et al. (1972) analyzed bacteria data from distribution water samples from 969 public water supplies in the U.S. They found that the frequency of detecting total and fecal coliforms by the MF procedure increased as the SPC levels increased to 500/ml, but decreased in frequency when SPC levls exceeded 1,000/ml. Their data appear in Table IV-5.

Sinclair (1984) collected 529 water samples from wells and distribution systems, and also found interference with coliform analysis on MF plates at SPC densities exceeding 500 colonies/ml. At densities above 500/ml, 18 of 37 samples produced overgrowth or the filters and caused false negative results. Anaerobic incubation of MF plates strongly suppressed non-coliform background gowth and enhanced coliform recovery by a factor of 2.2.

Clark (1980) examined microbial interference in the Presence-Absence (P-A) test be developed. He enumerated total coliforms in 3254 southern Ontario drinking water samples of both P-A test and the conventional MF procedure, using m-Endo agar LES. SPC levels were measured after 24 and 48 hours by a modified MF procedure (modified m-PCA) incubated at 35°C. Using SPC results after 48 hours, Clark found no apparent interference with coliform analysis with either test at SPC densities greater than 1000 colonies/ml (Table IV-6). Interference was demonstrated with the MF procedures, but not the P-A test, when SPC levels were greater than 1000 colonies/ml after 24 hours.

Table IV-4

Coliform Occurrences vs Standard Plate Count

	Standard Plate Count Range (per ml)		
	< 500	501-1000	> 1000
All Samples*	502	35	51
Samples with Coliforms** (MPN Method)	50	8	15
Percent of Samples	10%	22.9%	29.4%
MPN Range (per 100 ml)	2-49	5-79	2-920
MPN Mean (per 100 ml)	16	18	110
Samples with Coliforms*** (MF Method)	11	2	5
Percent of Samples	2.2%	5.7%	9.8%
MF Range (per 100 ml)	< 1-18	< 1-50	< 1-90
MF Mean (per 100 ml)	2	7	13
MPN % Occurrence less MF % Occurrence	7.8	17.2	19.6

* 588 of 613 samples had countable SPC
** 3 MPN positive samples did not have countable SPC
*** 1 MF positive samples did not have countable SPC

From Geldreich et al. (1978)

Table IV-5

Bacterial Plate Count vs. Coliform Detection in Distribution Water Networks for 969 Public Water Supplies

General Bacterial Population*		Total Coliform		Fecal Coliform	
Density Range per 1 ml	Number of Samples	Occurrences	Percent	Occurrences	Percent
<1-10	1013	47	4.6	22	2.2
11-30	371	28	7.5	12	3.2
31-100	396	72	18.2	28	7.1
101-300	272	48	17.6	20	7.4
301-500	120	30	25.0	11	9.2
501-1,000	110	21	19.1	9	8.2
>1,000	164	31	18.9	5	3.0
Total	2446	277	—	107	—

*Standard Plate Count (48 hrs. incubation, 35°C)
Geldreich et al., 1972.

Burlingame et al. (1984), in a laboratory study, examined the effects of pure cultures of four representative SPC bacteria on coliform colony sheen production on membrane filters. All four bacteria were isolated from a municipal water distribution system. A Flavobacterium sp. and a Bacillus sp., which failed to grow on m-Endo coliform medium, did not reduce the number of sheen colonies produced by either E. coli or Enterobacter cloacae, even when added in densities greater than 100,000/filter. Pseudomonas aeruginosa and Aeromonas hydrophila, which are able to grow on m-Endo medium, did interfere with sheen production. Statistical analysis indicated that 3000 P. aeruginosa per filter (30/ml) or 200 A. hydrophila per filter (2/ml) could reduce coliform counts. The investigators concluded this effect was due to overcrowding on the MF plate. Generally, MF plates containing more than 200 colonies are not countable and results are recorded as TNTC (too numerous to count). At the lowest challenge dose found to reduce sheen production, the 200 colony limit was exceeded. The investigators suggest that the coliform interference problem may be eliminated for coliform analyses using the MF procedure by strictly applying this 200 colony limit.

The results of this study contrast with the data from other studies mentioned in this section, where interferences were noted on MF plates with the total colony numbers below the 200 colony value. This difference may partially be due to coliform

injury induced by the SPC population before analysis, resulting in lower coliform counts. Moreover, differences in potency noted between the P. aeruginosa and A. hydrophila in the Burlingame et al., study suggest that other representatives of the SPC population may be sifficiently potent to reduce sheen colonies at densities well below the 200 colony limit.

In summary, these investigations demonstrate that high SPC densities (i.e., over 500/ml) can substantially interfere with both the MFT and MF tests, but that this phenomenon may not occur consistently. For the MF procedure, overcrowding on MF plates appears to be a major reason for atypical coliform colonies. Data indicate that this problem may be alleviated (but not eliminated) by anwerobic incubation of plates or a strict application of the 200 colony limit for filters.

Table IV-6

Percentage Frequency of Total Coliforms Recoveries Based On Two Procedures, and Detection at Various Density Ranges for Plate Count Occurrences

Count parameter	Counting Range (ml)	No. and percentage of positive samples detected by: Only MF tests[1] No.	%	Only P-A tests[2] No.	%	Only MF/P-A tests No.	%
24-h, plate count[3]	0	5	0.7	34	4.7	37	5.1
	1-100	118	5.6	223	10.5	251	11.9
	101-1000	52	21.0	68	27.4	80	32.3
	>1000	27	16.9	45	28.1	49	30.6
48-h, plate count[3]	0	—	—	3	1.6	3	1.6
	1-100	72	3.2	178	8.0	200	9.0
	101-1000	79	14.8	116	21.8	129	24.2
	>1000	51	16.6	73	23.8	85	27.2

[1]Membrane filtration procedure using m-Endo LES. Positive sample is 1 coliform/100ml.

[2]Presence-Absence procedure. Positive sample is a presence reaction

[3]m-Standard Plate Count Agar (modified), 35°C incubation

Source: Adopted from Clark (1980).

Presence-Absence Test

Because of the deficiencies of the current MF and MPN procedures, other media and procedures are being developed and evaluated. Clark (1968), for example, has developed a qualitative test for total coliforms and other selected organisms. In this test, 100 ml of water sample is added to 50 ml of 3X-broth in a single screw-cap culture bottle with an inverted tube, and incubated at 35°C for 24 or 48 hours. Acid and gas production indicate the presence of total coliforms. Clark (1982) reported that when strong acid and gas reactions were noted, coliforms were confirmed in over 93% of the cases. When any acid and gas were observed, total coliforms could be confirmed for 83% of the samples. One advantage offered by this type of qualitative test is the opportunity to analyze a larger number of samples per unit time than the conventional MPN test for the same price.

Clark has demonstrated the sensitivity and practicality of the Presence-Absence (P-A) test in a number of papers (Clark, 1968, 1969, 1980; Clark and Pagel, 1977; Clark et al., 1982). He has shown, for example, that coliform detection is higher with the P-A test than with the standard MF test (Clark, 1969). Preliminary, unpublished USEPA data support this. In the USEPA investigation, 1980 drinking water samples, some of which had been amended with small quantities of dilute sewage, were analyzed by the P-A, MPN, and MF procedures. Coliforms were detected in 58, 51, and 45 samples by the P-A, MPN, and MF tests, respectively.

In an investigation of over 100 municipal water systems using the P-A test along with subsequent identification procedures, Clark et al. (1982) found that Enterobacter aerogenes was usually the most commonly occurring coliform in drinking water. This was followed by either E. coli for systems using surface water or Aeromonas hydrophila for systems using groundwater.

Originally the P-A medium was modified MacConkey broth. More recently, because of difficulties in obtaining adequate supplies of this medium, Clark (1982) developed an alternative formulation consisting of lactose broth, lauryl tryptose broth, tryptone, and a dye. The new medium, adopted for use in routine analysis in 1979, performed in an equivalent manner to MacConkey broth with regard to the number of acid/gas reactions in presumptive tests (Clark, 1982). Until very recently, Clark used an inverted tube in the bottle to detect gas production, but now is evaluating the effectiveness of the test without these tubes (Clark, personal communications). Because of its simplicity and sensitivity in detecting coliforms, the Clark P-A test is tentatively scheduled for incorporation into the 16th edition of Standard Methods.

V. RISK ANALYSIS

Qualitative Data

There is widespread acceptance for using total coliforms as an indicator of drinking water quality. Coliforms do signal the presence of possible fecal pollution and are useful for indicating the presence of many, but not all, waterborne pathogens. They provide a reasonable measure of treatment efficiency and deterioration of water quality in the distribution system. Their continued use was supported by the microbiology workshop sponsored by the USEPA in 1981 (Olivieri, 1983).

There is ample evidence associating the presence of total coliforms with numerous waterborne disease outbreaks. Craun (1978a) examined outbreaks between 1971-1975, and found that in the majority of cases where sufficient coliform data were available, high levels of either total coliforms or fecal coliforms were present in water samples. Sobsey and Olson (1983) cite ten cases where pathogens implicated in an outbreak were found in the drinking water. Coliforms were detected in five of these cases and absent in one. Indicator presence for the other four cases was unknown. In the five coliform-positive waters, the pathogens included *Salmonella typhimurium*, *Shigella sonnei*, *Salmonella typhi*, enterotoxigenic *E. coli*, and *Giardia*. In the case where coliforms were not detected, the pathogen was *Giardia*.

A retrospective epidemiology study conducted by Petersen and Hines (1960) also associated total coliforms with waterborne

Table V-1

The Relation of Water Quality to Gastrointestinal Illness Incidence in 6 Rocky Mountain Communities

Community	Total People Surveyed	Total People Ill in June, July, and August	Overall Attack rate in June, July, and August (Percent)	Percentage of Water Sample Portions Pos. in June, July, and August
A	199	34	17.1	100.0
B	285	40	14.0	90.0
C	352	42	11.9	72.5
D	313	31	9.9	0.0
E	375	34	9.1	2.5
F	335	25	7.5	1.7

Source: Petersen and Hines, 1960

disease outbreaks. In this investigation, total coliform levels in the water supplies of six small Rocky Mountain communities were compared with the results of a morbidity survey performed in each community. The investigators asked about 100 families in each community what the family experience with gastrointestinal illness had been during the last three months (June to August 1957). The resulting data are shown in Table V-1. The six communities were then bifurcated into two categories, based on the coliform data. Communities with water supplies of high sanitary quality (A, B, and C, in the Table) had a gastroenteritis attack rate of 9%, while the other three communities suffered an attack rate of 14%. The difference was statistically significant ($P<0.01$), strong evidence that high levels of coliforms can reflect substantially higher levels of gastrointestinal disease.

Occasionally, total coliforms were not isolated from drinking water supplies associated with outbreaks. According to Craun (1978a), during 1971-1975 there were 5 outbreaks in which drinking water was epidemiologically implicated but where samples collected during the outbreak investigation were all negative for coliforms. In another review by Craun (1979), little or no bacterial contamination was reported during outbreaks of waterborne giardiasis. There are also instances in which Salmonella were isolated from water supplies in the absence or near absence of E. coli (Seligmann and Reitler, 1965). In this study, total colony counts were as high as 48,000/ml. According to Geldreich et al.

(1972), total counts as low as 500/ml may interfere with coliform detection.

One recently published study concluded that, even qualitatively, total coliforms are ineffective in signalling outbreaks. Batik et al. (1983), compared coliform records of 23 community water systems which reported outbreaks between 1971-1977 with those of selected comparison communities not reporting outbreaks. They also compared coliform results for 16 non-community water systems with outbreaks with 798 systems with no reported outbreaks. (A non-community water system is a public water system which provides less than year-round service (USEPA, 1976)). In both categories of systems, no statistical difference was observed, suggesting that coliforms were of limited predictive value. The investigators urged caution in interpreting these results because the sample size for outbreak communities was meager. Moreover, coliform data were collected from a period before the EPA regulations became effective, and thus inadequate monitoring conditions may have occurred. One-half of the community systems in the study were required to sample only twice a month or less; most of the non-community systems were sampled only once a year. In some cases, coliform monitoring was initiated only after an outbreak had occurred. This low monitoring frequency coupled with the non-randomness of coliform distribution patterns (Pipes and Christian, 1982) suggest that these coliform data are questionable.

Coliforms are often found in drinking water supplies in the absence of pathogens or outbreaks. This is nor surprising since some members of the coliform group are ubiquitous in the aquatic environment and not always associated with fecal contamination. Conversely, fecal pathogens or outbreaks may occur in the absence of total coliforms (Craun, 1978a). This may be due to interference of total coliform analysis by high densities of heterotrophic bacteria, inadequate coliform monitoring, or the inadequacy of total coliforms as an indicator for a particular pathogen.

In summary, the data in this section suggest that, in spite of occasional lapses, a waterborne disease outbreak of fecal contaminant origin is usually accompanied by the presence of total coliforms in the drinking water. The mere presence of coliforms, however, does not necessarily signify the concomittant presence of pathogens.

Quantitative Data

A few studies have examined the relationship between the densities of coliforms and selected pathogens in water. Kehr and Butterfield (1943) reported that heavily polluted rivers generally contained 1-100 *Salmonella typhi* organisms per 10^6 coliforms. Other investigators examining coliform: *Salmonella* ratios or coliform: enterovirus ratios in ambient water have reported orders of magnitude of variability (Dutka, 1973; Vaughn *et al.*, 1978). Thus a quantitative relationship between total coliforms and specific pathogens apparently does not exist in water.

Attempts to relate total coliform densities and the probability of waterborne disease outbreaks are almost nonexistent. An early attempt to quantify this relationship was conducted by Kehr and Butterfield (1943). They examined the Eberthella typhosa (Salmonella typhi)/coliform ratios in river water and sewage for several commuties (approximate range: 1-100 per 10^6 coliforms) and matched each ratio with the incidence of typhoid fever in that community. From these data, they developed a linear relationship between S. typhi per million coliforms vs. typhoid fever morbidity. Based on this and other data, the investigators estimated that between 1-2% of the population would develop typhoid fever by ingestion of a single S. typhi cell. This value and the S. typhi/coliform ratio data can be used to calculate a risk level for typhoid fever for a susceptible population. Unfortunately, the minimum infective dose value for S. typhi was apparently far too low (NAS, 1977). Moreover, the investigators state that widespread gastroenteritis accompanied even a few cases of typhoid fever.

The values for coliform density established by the U.S. Public Health Service for the 1942 and 1962 drinking water standards, and upon which the present USEPA (1976) standards are based, were apparently not based on quantitative data. Unfortunately, the rationale was never published, but it appears to have been based on what was cheaply and technically attainable (Dutka, 1973).

Table V-2

Summary Of Bacteriological Data From Water Systems Where Low Numbers of Total Coliforms Were Found In Tap Water Samples During the Outbreak Investigation[1]

CAUSE OF OUTBREAK:

I. Use of Untreated Water

1. 1/100 ml, 21/100 ml. TNTC
*2. (-),3/100ml, 3/100 ml, 8/100 ml, 19 100 ml, 32/100 ml
**3. (-), (-), 200/100 ml
4. (-), 2/100 ml, 3/100 ml, 6/100 ml, 10/100 ml, 13/100 ml
5. (-), (-), (-), 162/100 ml

II. Treatment Deficiency

1. (-), (-), 8/100 ml, 20/100 ml
†2. 15/100 ml
††3. <2.2 MPN
†††4. 12 samples all (-)
5. (-), 7200/100 ml
††††6. 4 samples (-), 3/100, 10/100 ml

III. Distribution Deficiency

1. (-)
2. (-)'s and low levels within DWS limits
3. 44 samples (-), 5/100, 8/100, 9/100, 10/100, 10/100, 26/100, >39/100 >39/100, 48/100, 52,100

IV. Miscellaneous Or Undetermined

1. 12/100, 22/100
2. (-)
3. 1/100, 1/100, 3/100, 4/100

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* 246/100 ml
** 100/100 ml
† 29/100 ml
†† >16 MPN
††† >50/100 ml
†††† >50/100 ml

[1]Outbreaks occurred between 1971-1975
Source: Craun, 1978a

There is some data relating coliform density with outbreaks (Table V-2), but they demonstrate that outbreaks are associated with densities ranging from zero to too high to count.

In summary, data in the literature does not demonstrate a quantitative relationship between coliform counts and either pathogen density or outbreak incidence.

Summary and Conclusions

The primary objective of microbiological monitoring is to minimize the potential presence of pathogens, and thus waterborne disease occurrence. Total coliform bacteria have been used for decades for this purpose, and the use of this group is widely accepted. Their use as a qualitative tool is scientifically supportable. Coliforms are usually present in fecally-polluted water and are normally detected during disease outbreaks (Craun, 1978a). Given their ubiquitous nature, however, coliforms are often found in the absence of fecal pollution. Moreover, there are reports of pathogen occurrence and disease outbreaks where coliforms were not detected (Craun, 1978a; Hejkal *et al.*, 1979; Payment, 1981). Nevertheless, total coliforms remain the best indicator of drinking water quality in spite of these and other limitations.

While the qualitative use of total coliforms can be scientifically justified, there is no scientific data in the literature supporting a particular value for coliform density, below which the

water can be considered safe. Pathogens and outbreaks have been associated with coliform densities ranging from zero to very high. This is not suprising: pathogens vary in their association with fecal contamination, their die-off times in water, and their resisting to disinfection. Although the total coliform indicator is an inexact tool, fecal pathogens in ambient water are usually accompanied by substantially higher levels of total coliforms. Since higher levels of pathogens should roughly translate to greater incidence of disease, coliform densities indirectly serve as a measure of waterborne disease incidence.

During the last five years (1978-1982), there have been 195 outbreaks and 49,049 cases of waterborne disease reported in the U.S. There is published evidence that these values represent substantial underestimates, primarily due to lack of recognition and lack of reporting of waterborne disease occurrence. Very low infective doses are associated with some waterborne disease agents (e.g., one particle for some viruses). For these reasons, pathogen presence in drinking water should be minimized. Treatment which provides coliform-free water should also reduce pathogens to minimal levels, even though large volume risk-free water is probably unattainable.

Thus the recommended no effect level for total coliforms is zero, i.e., no coliforms/100 ml on any EPA-approved analytical procedure. This presence-absence concept has several advantages

beyond minimizing pathogen presence. Sensitivity is improved because it is easier to detect coliform presence than to detect whether coliform densities fall below or above a specific value. The concept is much less sensitive to substantial changes in coliform density during transit/storage times after sample collection. In addition, data truncation implicit in analytical methodology is eliminated as a calculation difficulty (i.e., for the MPN procedure, data are truncated at 2.2 and 16 coliforms per 100 ml).

The NAS (1977) supports the use of total coliform, but does not address risk levels. They state that ". . . testing for coliform[s], while not perfect bacteriologically, is still the most reliable indicator of the possible presence of fecal contamination and therefore of the pathogens that may be present in water."

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